GENETIC UNIFORMITY OF EL ARBOL DEL TULE (THE TULE TREE)

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ABSTRACT

An electrophoretic analysis of enzymes was conducted on leaf material from each of eight major segments of the Tule Tree, a huge specimen of *Taxodium mucronatum* from Oaxaca, Mexico, variously interpreted as a single enormous tree, or as a natural grafting of several individuals. For comparison, two nearby conspecific individuals were also analyzed electrophoretically. The results are consistent with the hypothesis that the Tule Tree is one genetic individual. The literature on the Tule Tree is reviewed.

RESUMEN

Se llevó a cabo un estudio electroforético de enzimas en las hojas de cada uno de los ocho segmentos primarios del Arbol del Tule, un espécimen inmenso de *Taxodium mucronatum,* diversamente interpretado ya como un solo árbol enorme, o como producto de la fusión natural de varios árboles individuales. Para fines de comparación, dos ejemplares de la misma especie que crecían cerca, también fueron analizados por electroforesis. Los resultados obtenidos van de acuerdo con la hipótesis de que el Arbol del Tule es geneticamente un solo individuo. El artículo incluye una revisión de la literatura sobre esta extroaordinaria planta.

El Arbol del Tule, a Montezuma Bald-cypress or Ahuehuete (*Taxodium mucronatum* Ten. [Taxodiaceae]) growing in a churchyard at Santa María del Tule, Oaxaca, Mexico, is often cited as the largest individual tree in circumference in the world (Goetz et al. 1985; Johnston et al. 1988; Russell et al. 1987). The Guinness Book of World Records (Russell et al. 1987) gives its height as 135 ft (41.18 m) with a girth of 117.6 ft (35.84 m) five feet above the ground. According to the Encyclopaedia Brittannica (Goetz et al. 1985), basal circumference is 150 ft (45.75 m), if the bays and promontories of the buttressed trunk are followed. More than 20 men are reported necessary to encircle the trunk with outstretched arms (Johnston et al. 1988).

This tree, also known as El Gigante, has long been famous. Ac-

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cording to legend, this and two other large specimens were planted by a quetzalcoatl or prophet named Pecocha in the 6th century (Conzatti 1934). According to Martínez (1963), the first written reference to the tree is apparently that of Acosta (1590) who mentions a tree of enormous size three leagues from Oaxaca in New Spain. Cortes (Hora *in* Bateman et al. 1981) and Humboldt (Berry 1923; Lane 1953; Reyes 1884) or Bonpland (Reyes 1884) are reported to have seen the Tule Tree, with Lane (1953) stating that "Humboldt attached a plate to its massive trunk some 12 feet from the ground, whose inscription though partially overgrown by bark is still legible." Berry (1923) likewise cites Humboldt as having affixed a plate. Conzatti (1921) however, after an extensive search of the historical records, disputed such claims indicating there is no record of Cortes, Humboldt or Bonpland having seen the Tule Tree. Humboldt at least knew of the tree and discussed it in his Political Essay on the Kingdom of New Spain (1811) saying: "At the village of Santa María del Tule, three leagues east from the capital [Oaxaca], between Santa Lucia and Tlacochiguaya, there is an enormous trunc of cupressus disticha (sabino) of 36 metres in circumference. This ancient tree is consequently larger than the cypress of Atlixco, of which we have already spoken, the *dragonnier* of the Canary Islands, and all the boababs [baobabs] (Adansoniae) of Africa."

The age of the Tule Tree is also controversial, at least in part due to the question of whether the tree is composed of one or several genetic individuals. Menninger (1967) cited age estimates of 2000–4000 years, whereas Berry (1923) gave 4000–6000 years. Conzatti (1934) quotes the Enciclopedia Universal Europeo-Americana as follows: "Humboldt, who estimated their [trees at Santa María del Tule] age at four thousand years, says that they are larger than the biggest baobabs to be found in Africa. De Candolle supposes their age to be six thousand years." Lane (1952) likewise reported that "Humboldt guessed that its [Tule Tree] age might be anywhere between 4000 and 6000 years." However, Conzatti (1934), despite an extensive literature search, was unable to find any mention of the age of the tree in the writings of either Humboldt or De Candolle.

At the other extreme of age estimates, Bowers (1965) cites H. A. Dutton, who reportedly examined the tree repeatedly, as rating the tree as a multiple-trunk growth, probably about 500 years old. This low age estimate conflicts with the report by Acosta in 1590 of a tree of enormous size three leagues from Oaxaca. Little *in* Encyclopedia Americana 1987, while noting that some experts believe el Arbol del Tule to be the world's oldest tree with an age of 4000 years, states that others would give estimates as low as 1000 years if it developed from more than one individual.

Possibly the most reasonable age estimate is that of Conzatti (1934) who believed the tree to be not more than 2000 years old. He makes

this estimate based on the very rapid growth of individuals of the species, comparative growth of other conifers and particularly on a comparison of the growth rate of branches from a nearby conspecific individual. While indicating that his figures are given only as an indication, with extrapolation he arrived at a likely age of approximately 1500 years.

The highly irregular outline of the trunk has given rise to speculation that this massive specimen may represent a natural grafting together of several individuals (Anza *in* Humboldt 1811; Bandelier *in* Alvarez 1900; Conzatti 1934; Dutton *in* Bowers 1965; Hora *in* Bateman et al. 1981; Little *in* Encyclopedia Americana 1987), perhaps similar individually to the adjacent large cypresses known as "hijos del Tule" or "el hijo y el nieto" (Martínez 1963). Others however, believe that only one individual is represented (Bolaños 1857; Charnay *in* Alvarez 1900; Ramírez *in* Alvarez 1900; Reyes 1884). Confusion over the individuality of the tree has led to problems concerning age estimates and to questions over the status of the Tule Tree as the world's largest in circumference. Enzyme electrophoresis can potentially clarify the origin of this renowned specimen.

Materials and Methods

Plant material. Leaf samples were obtained from each of eight major segments (approximate compass directions: 0, 20, 70, 100, 160, 200, 220, 280 degrees) of the Tule Tree located at Santa María del Tule, approximately 9 km E of Oaxaca, Oaxaca, Mexico (*Diggs and Diggs 4065*). Leaf samples were also obtained from two other nearby large individuals ("hijos del Tule"). Vouchers have been deposited at BRIT and WS.

Electrophoresis. Leaf portions for electrophoresis were placed in plastic bags with wet paper towels and shipped to the laboratory. Electrophoretic procedures generally followed Soltis et al. (1983). Samples were prepared using the tris-HCl grinding buffer of Soltis et al. (1983) with 10% (wt/vol) PVP. Starch gel concentration was 12.5%.

The following enzymes were resolved: isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), phosphoglucoisomerase (PGI), phosphoglucomutase (PGM), 6-phosphogluconate dehydrogenase (6PGD), shikimate dehydrogenase (SkDH) and triosephosphate isomerase (TPI). LAP, PGI, and TPI were resolved on a modification of gel and electrode buffer system 8 of Soltis et al. (1983) as described by Soltis and Soltis (1987). IDH, MDH, PGM, 6PGD and SkDH were resolved using system 9 of Soltis et al. (1983). Staining for all enzymes followed

Soltis et al. (1983) except LAP; staining for LAP followed Soltis and Soltis (1987).

Results and Discussion

Enzyme electrophoresis resulted in clear staining for eight enzymes encoded by 16 putative loci: *Idh, Lap, Mdh-1, Mdh-2, Mdh-3, Mdh-4, Mdh-5, Pgi-1, Pgi-2, Pgm-1, Pgm-2, Skdh-1, Skdh-2, 6pgd, Tpi-1,* and *Tpi-2.* The observed bands migrated anodally for all enzymes examined.

The number of isozymes observed for the enzymes examined is consistent in most instances with reports for other seed plants (Gilchrist and Kosuge 1980; Weeden and Gottlieb 1980; Gottlieb 1981, 1982). However, for 6PGD only one isozyme was observed, whereas two isozymes would be expected in diploid seed plants (Gottlieb 1981, 1982). The presence of only one isozyme when two are expected could be due to: 1) comigration of the two isozymes, or 2) the loss of expression of one isozyme, perhaps as a result of the length of time involved in transporting the material from the field to the laboratory. In support of the latter hypothesis is the observation that one of the two observed isozymes was only faintly stained for PGI, PGM, and TPI.

Idh, Lap, Mdh-1, Mdh-2, Mdh-3, Mdh-4, Mdh-5, Pgi-1, Pgi-2, Pgm-1, Pgm-2, Skdh-1, 6pgd, Tpi-1, and *Tpi-2* were monomorphic, with all samples of the Tule Tree, as well as the two neighboring trees sampled, displaying bands of identical electrophoretic mobility. The findings for these monomorphic loci are in agreement with the idea that the Tule Tree is a single individual, but are equivocal given that the neighboring trees displayed the same allozymes. Significantly, all samples of the Tule Tree were heterozygous for *Skdh-2,* exhibiting *Skdh-2ab.* In contrast, both neighboring trees possessed only *Skdh-2b.* The fact that all eight samples of the Tule Tree were heterozygous for *Skdh-2,* whereas both neighboring trees were homozygous, provides the strongest evidence in support of the hypothesis that the Tule Tree originated from only a single seedling.

Conclusions

More data on the amount of genetic variation to be expected in the species and on the occurrence of natural grafting would enhance a judgment as to the origin of this tree. However the genetic data are in agreement with the hypothesis that only one genetic individual is involved. Most noteworthy is the fact that all samples from the Tule Tree displayed the same heterozygous phenotype for *Skdh-2,* in contrast to the homozygosity of the nearby trees. Our results further strengthen the traditional interpretation of El Arbol del Tule as a truly exceptional individual.

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